In vivo assessment of heart function under chronic hypoxic stress with volumetric optoacoustic tomography

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Abstract: Chronic hypoxia in pulmonary diseases is known to have a severe negative impact on heart function, including right heart hypertrophy, increased workload on the heart and arrhythmia. Yet, the direct effect of the chronic hypoxic environment on the cardiovascular system is still not fully understood. Usual pre-clinical analytic methods analysing this effect are limited to ex vivo histology or highly invasive approaches such as right heart catheterisation, which inevitably interfere with cardiac tissue. In this work, we propose volumetric optoacoustic tomography as a method for assessing heart function in response to chronic hypoxia non-invasively. Hypoxic and normoxic murine hearts were imaged in vivo at high temporal (100 Hz) and spatial resolution (200 m). Analysis of the murine models on a beat-to-beat scale enabled identifying and characterizing arrhythmic events in hypoxic models. In addition, blood flow was tracked using indocyanide green (ICG) contrast agent, which revealed a clear difference in the pulmonary transit time (PTT) between the hypoxic and normoxic models. Validation for presence of hypoxia in the lungs was carried out by -smooth muscle actin staining for muscularization of the pulmonary vasculature. We expect that the novel capabilities offered by volumetric optoacoustic tomography for analysing impaired heart function under hypoxic conditions in pre-clinical models will provide important insights into early diagnosis and treatment methods for pulmonary diseases.

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ABSTRACT

Chronic hypoxia in pulmonary diseases is known to have a severe negative impact on heart function, including right heart hypertrophy, increased workload on the heart and arrhythmia. Yet, the direct effect of the chronic hypoxic environment on the cardiovascular system is still not fully understood. Usual pre-clinical analytic methods analysing this effect are limited to *ex vivo* histology or highly invasive approaches such as right heart catheterisation, which inevitably interfere with cardiac tissue. In this work, we propose volumetric optoacoustic tomography as a method for assessing heart function in response to chronic hypoxia non-invasively. Hypoxic and normoxic murine hearts were imaged *in vivo* at high temporal (100 Hz) and spatial resolution (200 μm). Analysis of the murine models on a beat-to-beat scale enabled identifying and characterizing arrhythmic events in hypoxic models. In addition, blood flow was tracked using indocyanide green (ICG) contrast agent, which revealed a clear difference in the pulmonary transit time (PTT) between the hypoxic and normoxic models. Validation for presence of hypoxia in the lungs was carried out by α-smooth muscle actin staining for muscularization of the pulmonary vasculature. We expect that the novel capabilities offered by volumetric optoacoustic tomography for analysing impaired heart function under hypoxic conditions in pre-clinical models will provide important insights into early diagnosis and treatment methods for pulmonary diseases.

1. INTRODUCTION

Pulmonary disease is one of the leading causes of mortality worldwide, following closely after cardiovascular disease which continuously holds highest mortality rates [1]. Chronic hypoxic conditions in the lung affect normal physiological processes, including heart function. Chronic hypoxia, which may be caused by chronic obstructive pulmonary disease (COPD) and high altitudes for non-native dwellers may have severe outcomes such as pulmonary vasoconstriction caused by the muscularization of otherwise non-muscular arteries contributing to pulmonary heart disease (PHD) [2-4].

Current clinical methods of detection include Ultrasound (US) and Magnetic Resonance Imaging (MRI) for evaluation of right ventricular (RV) hypertrophy and right atrial (RA) dilation; Electrocardiogram (ECG) for RV

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hypertrophy and arrhythmia; and RV catheterization for pressure sensing [5-6]. In pre-clinical studies, methods of assessing heart function in PHD are limited to histopathological ex vivo analyses or highly interfere with the integrity of the heart tissue. To our knowledge, non or minimally invasive imaging and assessment of pre-clinical models of hypoxia has not yet been established.

Optoacoustic (OA, photoacoustic) is becoming a powerful tool in pre-clinical imaging and is highly suited to *in vivo* cardiac imaging due its high spatial and temporal resolution, indigenous and exogenous optical contrast and a deeper visualization of the heart in a non-invasive manner. Recent developments in OA imaging has allowed for 3D volumetric and real-time (beat to beat scale) imaging of the mouse heart, overcoming the limitations of 2D imaging [7]. The non - invasive nature of OA imaging ensures an intact heart throughout imaging and analysis, thus offering a more accurate overview on heart function. In this work, we demonstrate a method in which *in vivo* heart function is analyzed non-invasively in chronic hypoxic murine models with an aim to deepen our understanding of the effects on the circulatory system in response to an impaired pulmonary system.

2. METHODS

2.1 Chronic Hypoxic Model and Handling:

All animal experiments were approved and conducted under the local regulations (Government of Upper Bavaria Munich and Helmholtz Centre Munich under animal protocol reference number 55.2-1-2532-50-12). Mice (129S/Sv/C57BL6 mixed background) were divided into two groups, namely hypoxic (10% oxygen for 21 days, n=5) and normoxic/control (n=5) (Fig 1A). Induction of hypoxia as a disease model of chronic hypoxic conditions was based on a previously described method [8]. Mice were anesthetised with 2% isoflurane – oxygen medical mix (~0.81 l/min gas flow) for *in vivo* heart imaging and placed on top of the OA imaging probe filled with a solid clear agar matrix (3w/v% conc.). 100 nmol of FDA-approved indocyanine green (ICG, Profiplus Bvba, Kortessem, Belgium) diluted in 50 µl saline solution was injected intravenously after the beginning of the image acquisition procedure.

2.2 OA Imaging of Heart Function:

High-frame-rate images of the beating mouse heart were acquired with a volumetric optoacoustic tomography (vOT) system specifically designed for high quality pre-clinical cardiac imaging (Fig.1 B) [7][9] A detailed description of the vOT imaging set up has been previously described in detail [7]. In short it is composed of a spherical array transducer (Imasonic Sas, Voray, France) (Fig. 1B), where the heart was illuminated (800nm at sampling frequency 100Hz) with a fast-tuning pulsed laser (Innolas Laser GmbH, Krailling, Germany) guided via a fibre bundle (Ceram Optec GmbH, Bonn, Germany), where OA signals were sampled by the data acquisition system (Falkenstein Microsysteme GmbH, Taufkirchen, Germany). The wavelength corresponds to the absorbance peak of ICG. OA data was acquired for each ICG injection and 3D images were reconstructed in a volume of 12x12x12 mm^3 (120x120x120 voxels) using the back-projection method (Fig. 1 C) [10].

2.3 PTT & heart beat analysis:

The method for extracting the pulmonary transit time (PTT) values from the heart is described in detail in previous work [7]. In short, the PTT was measured as the difference between the maximum signal peaks between the right and left ventricles, corresponding to the time of appearance of the ICG bolus (Fig. 2A). The PTT was measured for each ICG injection for hypoxic and normoxic models and heart waveforms from different structures of the heart including the right atrium (RA), right ventricle (RV), left ventricle (LV) and aorta were extracted from images (Fig 2A). Abnormal beating events were identified when a single heartbeat length was significantly longer than other heartbeats rendering a normal periodic rhythm.

2.4 Lung Remodelling:

Lungs were isolated and stained with alpha-smooth actin for the presence of small muscularized vessels for validation that chronic hypoxic conditions were present in the murine models.

3. RESULTS

3.1 Real-time tracking of blood flow:

The blood flow within the pulmonary circuit of the mice was tracked with ICG injection. Since the absorption peak of ICG is approximately at 800nm, the use of this wavelength was employed in order to visualise the dye clearly. The blood was tracked within one heartbeat from entry into the right heart and re-entry into the left heart and aorta, after completion of the pulmonary circuit (Fig. 2A). The OA signal intensity was plotted across 12 seconds (1200 frames) of a voxel within the RV, LV and aorta. The curves were plotted before, during and after ICG injection in order to track the change in OA signal intensity, correlating to the blood flow, at different locations in the heart (Fig. 2A). The PTT was measured as the time it takes the ICG bolus (or blood) to enter the right heart, travel through the lungs and return to the left heart. The entry times of the ICG can be visualised as the maximum OA signal intensities within the extracted curves.

3.2 PTT analysis and irregular heartbeat detection:

The PTT was measured for all hypoxic and normoxic models as described in the methods (Fig 2B). A t-test was carried out in order to analyse the difference between the PTT values of the models. The PTT values measured from the hypoxic models (mean 1.91s) were significantly longer than the normoxic models (mean 1.43s; $P < 0.0026$). The PTT suggest the time taken for the heart to deliver blood through the pulmonary circuit is significantly longer in hypoxic models.

After image reconstruction of 2000 frames with vOT, irregular heart beating was clearly identified within the hypoxic models, whereas only regular beating was visualised in normoxic models. Waveforms of the OA signal intensity over time presented clear abnormalities; where heartbeats of normal length were interrupted with larger beating periods (Fig. 3A). The disturbed heart beats take a significantly longer time to complete one heartbeat ($P < 0.0001$) (Fig 3B). The trends in the beating mouse heart were identified due to the 100Hz temporal resolution of vOT.

3.3 Lung remodelling:

Increased muscularization of vessels was present in lungs under hypoxic conditions. A clear difference was found between the hypoxic and normoxic models ($p < 0.01$), where the number of muscularized vessels was greater in hypoxic lungs compared to normoxic lungs (Fig. 4). The histopathological results were used as validation for the presence of hypoxia in the murine models.

3. DISCUSSION

We have studied the potential of vOT for assessing *in vivo* heart function in murine models in response to chronic hypoxic conditions and have shown that the heart function of hypoxic models was significantly impaired in response to the reduced oxygen intake by analysing the pulmonary transit time and heartbeat irregularities. So far, pre-clinical assessment of PHD has been often limited to *ex vivo* or highly invasive procedures that interfere with the integrity of the heart tissue, thus an overall accurate perception of heart function in living organisms remains inaccessible.

The PTT in hypoxic models was considerably longer than in normoxic models, indicating deteriorated heart function in response to hypoxic conditions. This parameter has previously been shown to serve as an accurate indicator of heart performance under pathophysiological conditions in the murine heart [11]. The significantly longer PTT in the hypoxic models strongly suggests an impaired pulmonary circuit within hypoxic mice. The narrowed pulmonary vasculature, a result of chronic hypoxic conditions, likely increases resistance of blood flow from the heart, causing it to work harder while also weakening the cardiac muscle. Histopathological studies of vessel muscularization in the lungs is generally considered the gold standard for evaluation of presence of hypoxia in the lungs. However, analysis of vessel muscularization is limited to *ex vivo* studies, while vOT can offer an alternative method of analysis of *in vivo* heart function.

Arrhythmic events resulting from hypoxic conditions (including COPD) are well documented in humans [12]. Irregular heart beating within the hypoxic mice models could be detected with vOT. The waveforms extracted *in vivo* from the heart using vOT followed similar outlines of pressure waveforms that are usually extracted by catheterisation [13]. Such waveforms easily detect changes in the heart beat rhythm corresponding to abnormalities in the cardiac cycle.

In conclusion, the PTT and heart beat rhythm were both altered in hypoxic hearts with respect to normoxic hearts. Herein, we have focused specifically on the response of the heart under hypoxic conditions by examining the whole murine heart directly in three dimensions, therefore providing an accurate representation of heart function. *Ex vivo* analysis of the lungs validated the presence of hypoxia, suggesting that both heart and lung analysis must be performed to properly study the effects of hypoxic conditions. Overall, vOT has been suggested as a method offering new capabilities for studying *in vivo* heart function under physiological stress without compromising the integrity of the heart, which is not possible with existing approaches.

FIGURES

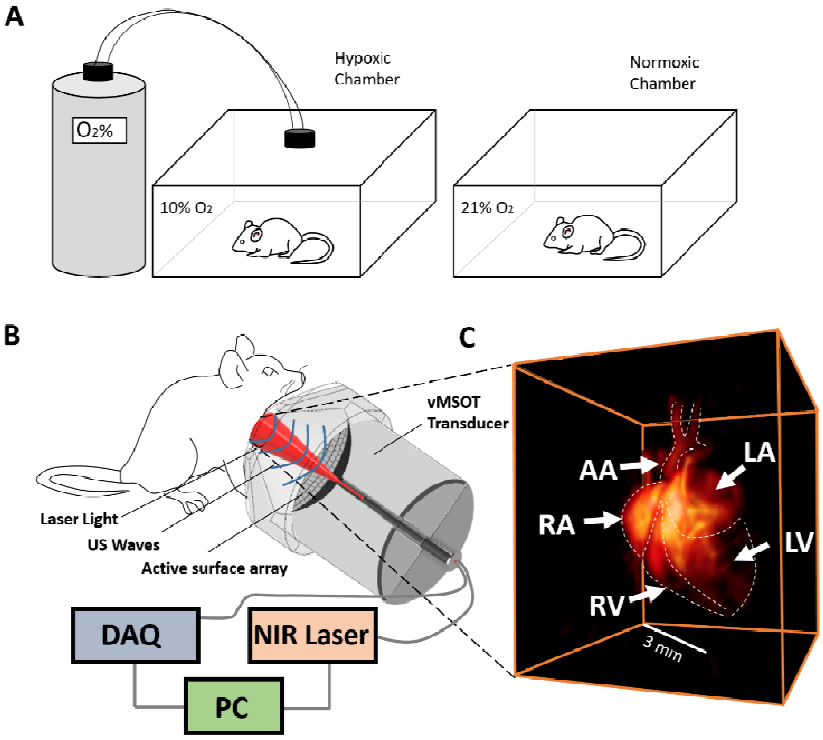

Figure 1: The experimental set up. (A) The hypoxia chamber with 10% oxygen monitored by the control system and normoxic chamber with regular environmental conditions (21% oxygen). **(B)** Non-invasive scanning procedure of the murine heart with the volumetric multrespectral optoacoustic probe (vOT). (US; ultrasound, NIR; near-infrared, PC; personal computer DAQ; data acquisition system). **(C)** 3D view of the reconstructed vOT image of the whole murine heart (AA; aortic arch, RA; right atrium, LA; left atrium, RV; right ventricle, LV; left ventricle).

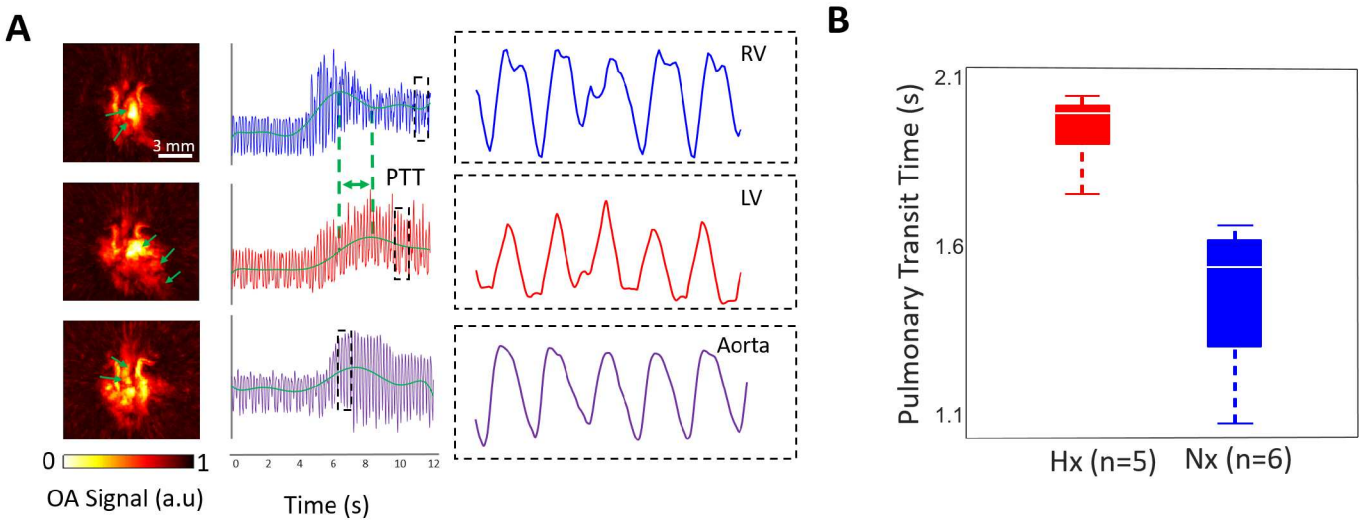

Figure 2: PTT (A) Blood flow tracking with ICG through a single heartbeat with PTT and waveform extraction (PTT; pulmonary transit time, RV; right ventricle, LV; left ventricle). **(B)** A box plot representing the acquired PTT values measured in normoxic and hypoxic mice, where a significant difference ($p < 0.0026$) was measured (Nx; normoxic, Hx; hypoxic

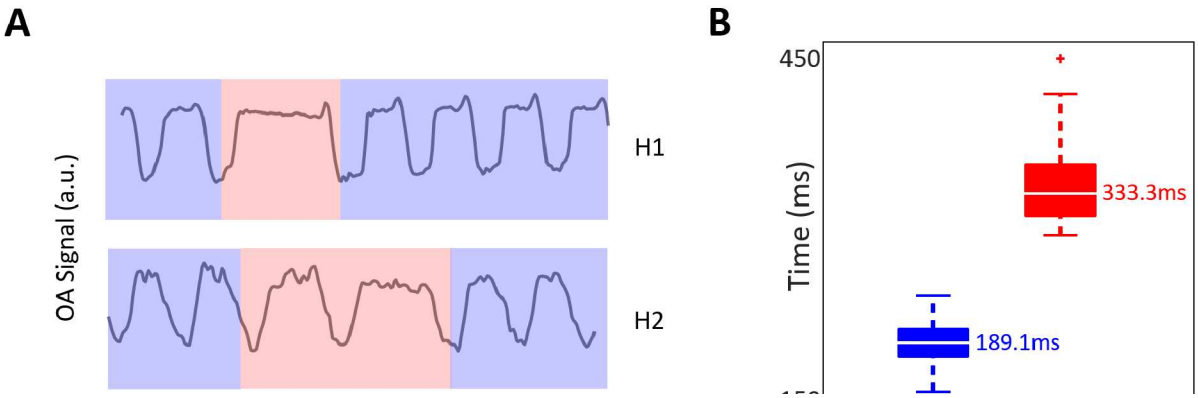

Figure 3: Abnormal beat characterisation (A) Plots of heart beat cycles in 2 hypoxic, where normal beats are highlighted in blue and irregular beats are highlighted in red (H; Hypoxic mouse **(B)** A box plot representing differences in time of normal beating cycles (mean 189.1ms per beat) and abnormal beating cycles (mean 333.3ms per beat), where significant difference was measured ($P < 0.00001$) (NB; normal beating, AB; abnormal beating).

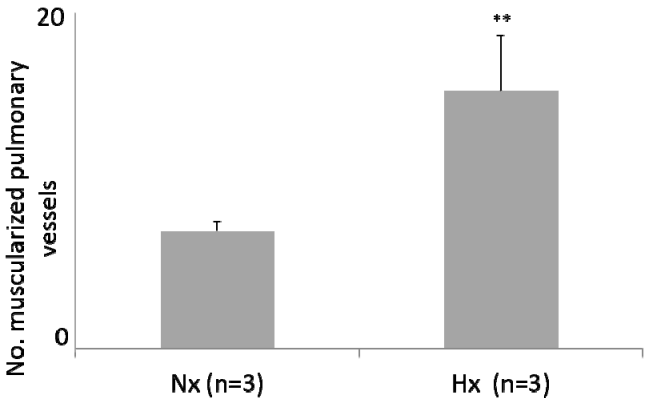

Figure 4: Lung Remodelling: SMActin staining of murine lungs , where there is significantly more ($p<0.01$) muscularized small vessels in hypoxic lungs compared to normoxic lungs (Nx, normoxic, Hx, hypoxic).

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